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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: Le A 33 256

U S APPLICATION NO (If known, see 37 CFR 1 5): 09/830741

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/08181	28 October 1999 (28.10.99)	30 October 1998 (30.10.98)

TITLE OF INVENTION
PHOSPHINATE PEPTIDE ANALOGS FOR THE TREATMENT OF FIBROTIC DISORDERS

APPLICANT(S) FOR DO/EO/US
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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is attached hereto (required only if not communicated by the International Bureau).
   b. [ ] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [X] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [X] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information:
    1) **Certificate of Mailing under 37 C.F.R. 1.10;**
    2) **Transmittal of Information Disclosure Statement under 37 C.F.R. 1.97(b);**
    3) **Information Disclosure Citation (Modified Form PTO-1449) and references cited therein**
    4) **Return Receipt Postcard.**

Date of Deposit: 30 April 2001
Express Mail Label No.: EK662544750US

U.S. APPLICATION NO (if known, see 37 CFR 1.5) 09/830741	INTERNATIONAL APPLICATION NO PCT/EP99/08181	ATTORNEY'S DOCKET NUMBER Le A 33 256

21. [X] The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ **860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	**4** - 20 =	**0**	x **$18.00**	$ **0.00**	
Independent claims	**0** - 3 =	**0**	x **$80.00**	$ **0.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)	**0**		+ **$270.00**	$ **0.00**	
TOTAL OF ABOVE CALCULATIONS =				$ **860.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$	
SUBTOTAL =				$ **860.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ **860.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ **860.00**	
				Amount to be refunded:	$
				charged:	$

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. **13-3372** in the amount of $ **860.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **13-3372**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

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41,670
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Phosphinate-peptide analogs for treating fibrotic diseases

The invention relates to the use of phosphinate-peptide analogs, as inhibitors of procollagen C-proteinase (PCP), for treating fibrotic diseases.

It is known that procollagen C-proteinase (PCP) is a key enzyme in fibrogenesis. It catalyzes the hydrolytic elimination of the procollagen propeptides from procollagens I, II, III and IV and also laminin V [cf. Amano S, Takahara K; Gerecke D, Nishiyama T, Lee S, Greenspan DS, Burgeson RE: Bone morphogenetic protein-1 is the processing enzyme for laminin 5 in human keratinocytes, Mol. Biol. Cell 7 (suppl.) 58A (1996)]. Consequently, PCP is a key enzyme in collagen processing [cf. Olsen BJ: Morphogenesis: collagen it takes and bone it makes, Curr. Biol. 6: 645-647 (1996)]. It was demonstrated in BMP-I knock-out mice that complete absence of PCP leads to incomplete collagen processing with the deposition of atypical, loose collagen fibrils [cf. Suzuki N, Labosky PA, Furata Y, Hargett I, Dunn R, Fogo AB, Takahara K, Peters DM, Greenspan DS, Hogan BL: Failure of ventral body wall closure in mouse embryos lacking a procollagen C-proteinase encoded by BMP-1, a mammalian gene related to Drosophila tolloid, Development 122: 3587-3595 (1996)].

PCP is probably also responsible for the hydrolytic elimination of the propeptide sequence of lysyl oxidase. The elimination of the prosequence probably leads to the activation of the catalytic lysyl oxidase activity of the mature form [cf. Pachenko MV, Stetler-Stevenson WG, Trubetskoy OV, Gacheru SN, Kagan HM: Metalloproteinase activity secreted by fibrogenic cells in the processing of prolysyl oxidase. Potential role of procollagen C-proteinase, J. Biol. Chem. 271: 7113-7119 (1996)]. Active lysyl oxidase covalently links opposing collagen fibrils to each other. In this way, PCP also indirectly increases the biological stability of the collagen towards degradation by collagenases.

PCP, or closely related proteins, appear also to play a role in the release of TGFβ-type growth factors. Recent studies have shown that PCP-like proteases are able to liberate TGFβ-type growth factors from an inactive complex with TGFβ-binding proteins [cf. Marques G, Musacchio M, Shimell MJ, Wünnenberg-Stapleton K, Cho KWY, O'Connor MB: Production of a DPP activity gradient in the early drosophila embryo through the opposing actions of the SOG and TLD proteins, Cell 91: 417-426 (1997); Blader P, Rastegar S, Fischer N, Strähle U: Cleavage of the BMP antagonist chordin by zebrafish tolloid, Science 278: 1937-1949 (1997)]. In this case, the binding partner for the TGFβ-type growth factors is decomposed by means of specific proteolysis. Consequently, PCP may possibly also indirectly possess a TGFβ-type agonistic activity. PCP can therefore be ascribed a crucial role in fibrogenesis.

PCP activity has its origin in splicing variants of the BMP-I gene [cf. Kessler E, Takahara K, Biniaminow L, Brusel M, Greenspan DS: Bone morphogenetic protein-1: The type I procollagen C-proteinase, Science 271: 360-362 (1996)); Reddi AH: BMP-1: Resurrection as procollagen C-proteinase, Science 217: 463 (1996); Li SW, Sieron AL, Fertala A, Hojima Y, Arnold WV, Prockop DJ: The C-proteinase that processes procollagens to fibrillar collagens is identical to the protein previously identified as bone-morphogenetic protein-1. Proc. Natl. Acad. Sci. USA 93: 5127-5130 (1996)]. It is so far definitely known that splicing variants BMP-I-I and BMP-I-III (tld variant) are able to cut procollagen and pro-lysyl oxidase specifically. While relatively recent studies have identified further BMP-1 splicing variants, the biological function and substrate specificity of the latter have not yet been fully elucidated [cf. Janitz M, Heiser V, Böttcher U, Landt O, Lauster R: Three alternatively spliced variants of the gene coding for the human bone morphogenetic protein-1. J. Mol. Med. 76: 141-146 (1998)].

Although success has so far only been achieved in expression-cloning and purifying PCP in small yields, a large number of structural details of the enzyme are known. Thus, PCP belongs to the family of astacin proteases. The crystal structure of astacin

is known in detail. There is a very high degree of structural homology between the catalytic domain of BMP-I and astacin such that it has been possible, on the basis of this homology, to assign to many amino acids in the PCP protease domain their probable structural and biochemical function [cf. Stöcker W, Gomis-Rüth FX, Bode W, Zwilling R: Implications of the three-dimensional structure of astacin for the structure and function of the astacin family of zinc-endopeptidases, Eur. J. Biochem. 214: 215-231 (1993)].

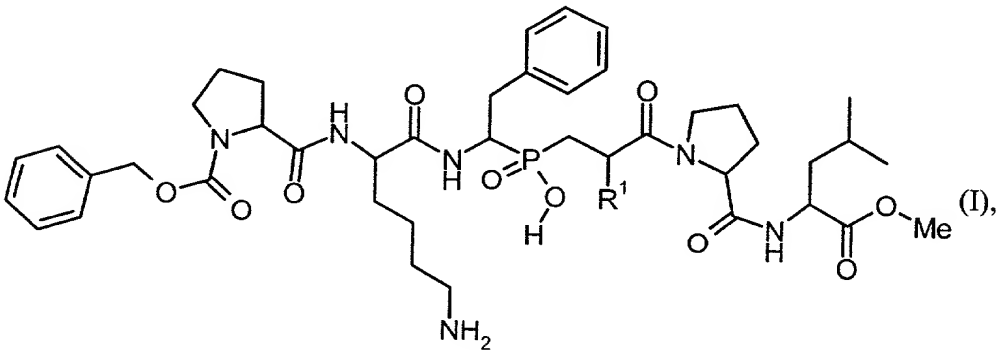
In the past, it has been possible, by means of computer-guided molecular modeling, to infer the binding of substrate to the active center of the astacins in molecular detail [cf. Stöcker W, Grams F, Baumann U, Reinemer P, Gomis-Rüth FX, McKay DB, Bode W: The metzincins – Topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-endopeptidases, Prot. Sci.: 823-840 (1995)]. These studies led to the rational design of phosphinate-peptide analogs which inhibit astacin with a high degree of potency. The complex between a phosphinate inhibitor and astacin has been structurally elucidated (cf. Grams F, Dive V, Yiotakis A, Yiallouros I, Vassilou S, Zwilling R, Bode W, Stöcker W: Structure of astacin with transition-state analogue inhibitor, Nature Struct. Biol. 3: 671-675 (1996)].

Despite the high degree of structural homology between astacin and the catalytic domain of BMP-1, it has so far been assumed that, because of biochemical differences with regard to their reaction behavior and also in their ability to be inhibited by protease inhibitors, the two proteases are in fact markedly different. For example, there are biochemical differences between astacin and BMP-I with regard to their substrate specificity (as a crayfish digestive enzyme, astacin hydrolyzes collagen-like proteins relatively nonspecifically whereas PCP cuts highly specifically at only one site in the procollagen molecule and in pro-lysyl oxidase).

To date, only low-potency inhibitors of PCP, to which an antifibrotic effect is attributed, have been described in the literature [cf. Brenner M, Ho WB: C-proteinase

inhibitors for the treatment of disorders related to the overproduction of collagen. WO 97/05865].

It has now been found, surprisingly, that phosphinate-peptide analogs of the general formula (I),



(I),

in which

R^1 represents hydrogen or methyl,

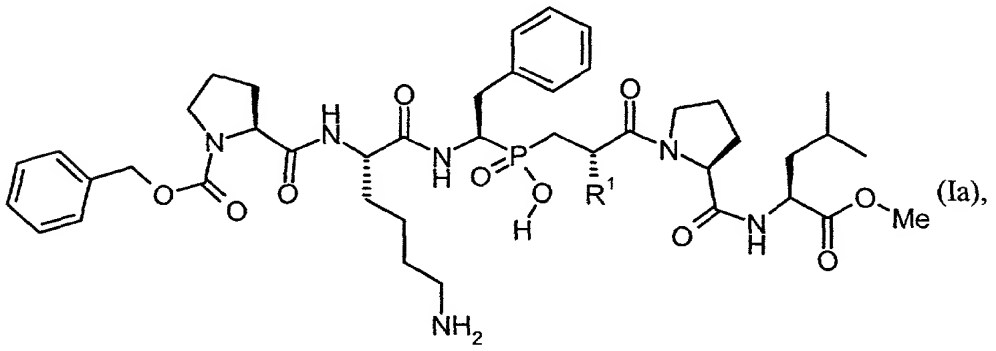
and their salts and isomers inhibit PCP with a very high degree of potency and can therefore be used for the treatment and prophylaxis of fibrotic diseases.

Within the context of the invention, preference is given to physiologically harmless salts. In general, physiologically harmless salts are salts of the compounds according to the invention with inorganic or organic acids. Preference is given to salts with inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid or sulfuric acid, or salts with organic carboxylic or sulfonic acids, such as acetic acid, maleic acid, fumaric acid, malic acid, citric acid, tartaric acid, lactic acid, benzoic acid, or methanesulfonic acid, ethanesulfonic acid, phenylsulfonic acid, toluene-sulfonic acid or naphthalenedisulfonic acid.

The compounds according to the invention can exist in stereoisomeric forms which either relate to each other as image and mirror image (enantiomers) or which do not relate to each other as image and mirror image (diastereomers). The invention also relates to the antipodes and to the racemic forms as well as to the diastereomeric mixtures.

The compounds of the general formula (I) can be present in all the enantiomeric and diastereomeric forms. Preference is given to those isomers in which the parts of the molecule formed from proline, lysine and leucine possess the L configuration, as well as to their salts and prodrugs.

Particular preference is given to using phosphinate-peptide analogs of the general formula (I), which have the configuration as shown,



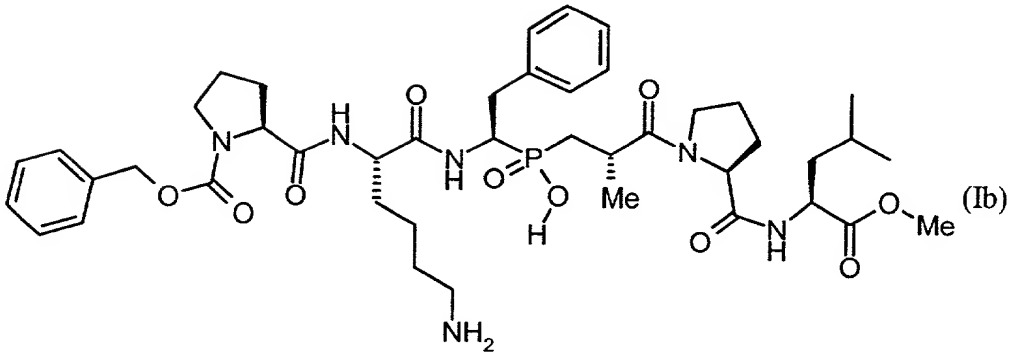
(Ia),

in which

R^1 represents hydrogen or methyl,

and their salts and isomers for the treatment and prophylaxis of fibrotic diseases.

Very particular preference is given to using the compound of the general formula (Ib)



(Ib)

designated **Z---PKF(PC)APL---O-Me** in that which follows,

and its enantiomers and its salts for the treatment and prophylaxis of fibrotic diseases.

The compounds of the general formula (I) are known and can in principle be prepared from corresponding phosphinate compounds using customary methods of peptide synthesis [cf., in this regard, Yiotakis A, Vassilio S, Jiracek J, Dive V: Protection of the Hydroxyphosphinyl Function of Phosphinic Dipeptides by Adamantyl. Application to the Solid-Phase Synthesis of Phosphinic Peptides, J. Org. Chem. 61: 6601-6605 (1996); Campagne JM, Coste J, Guillou L, Heitz A, Jouin P: Solid phase synthesis of phosphinic peptides, Tetrahedron Lett. 34: 4181-4184 (1993)].

The results were unexpected because BMP-I, meprin and astacin differ from each other, in particular, in the so-called S1' loop. A sector from this loop is depicted here:

astacin:	T--DPYD
BMP-I:	KPPIG-Q
meprin:	I--IG-Q

This loop forms the essential part of the S1' binding pocket of the astacin-like proteases. It is here, as has been shown using the example of astacin and meprin, that the key to the differing substrate and inhibitor specificities of the two enzymes lies. Meprin and astacin differ very markedly from each other in the S1' pocket. PCP additionally has two proline residues and one lysine residue in this region. As has been shown by computer modeling the protease domain of PCP, the lysine residue is very probably involved in binding the carboxyl group in the side chain of the aspartate in P1' next to the cleavage site in the procollagen. According to the model, the proline residues are in the *cis* configuration and represent a peculiarity of the BMP-I subfamily among the astacins. By contrast, astacin does not cleave next to acidic residues. For these reasons, it was completely unexpected that a phosphinate which was designed for inhibiting astacin [cf. Yiallouros I, Vassiliou S, Yiotakis A, Zwilling R, Stöcker W, Dive V: Phosphinic peptides, the first potent inhibitors of astacin, behave as extremely slow-binding inhibitors, Biochem. J. 331: 375-379 (1998)] would prove to be an effective PCP inhibitor.

The compounds of the general formula (I) according to the invention exhibit a valuable pharmacological activity spectrum which it was not possible to foresee.

Surprisingly, the compounds according to the invention are notable for having a very high potency when inhibiting PCP.

The compounds according to the invention are therefore suitable for treating liver fibroses of any genesis and fibroses which are manifested in other organs.

These fibroses include various groups of diseases which are accompanied by a qualitative change in collagen production or by an increased deposition of collagen in the extracellular space, such as liver fibroses of differing origin, such as alcoholic liver cirrhosis, biliary cirrhosis, hepatitis of viral or other genesis, idiopathic interstitial fibroses, idiopathic lung fibroses, acute pulmonary fibroses, acute respiratory distress syndrome (ARDS), perimuscular fibroses, pericentral fibroses,

dermatofibromas, kidney fibroses, diabetic nephropathy, glomerulonephrites, systemic or local scleroderma, keloids, hypertrophic scar formation, joint adhesions, arthroses, myelofibroses, cicatrization of the cornea, cystic fibrosis, muscular fibroses, Duchenne's muscular dystrophy, strictures of the esophagus, Ormond's disease, Crohn's disease, ulcerative colitis and aneurysms of the large vessels.

In addition, the invention encompasses fibrotic diseases which are initiated or provoked by surgical scar revisions, plastic surgery, glaucoma, cataract fibroses, cicatrizations of the cornea, graft-versus-host disease, surgical interventions performed on tendons, nerve trapping syndromes, Dupuytren's contracture, adhesions resulting from gynecological interventions, pelvic adhesions, epidural fibroses, and diseases of the thyroid gland or the parathyroid glands, and also by metastatic bone invasion, by multiple myeloma or by restenoses.

Detecting PCP activity in a fluorescence dequenching test

For the purpose of detecting PCP activity, a synthetic decapeptide substrate having the sequence

DABCYL – Asp – Phe – Tyr – Arg – Ala – Asp – Gen – Pro – Arg – Asp (EDANS) – NH_2

was cleaved by adding PCP. This peptide frequency corresponds to the region in procollagen α_2(I) which is cleaved by PCP. The cleavage sequence is known to biochemical experts [cf. Lee ST, Kessler E, Greenspan DS: Analysis of site-directed mutations in human pro-α_2(I) collagen which block cleavage by the C-proteinase, J. Biol. Chem. 265: 21992-21996 (1990)], as is the dequenching test method [cf. Matayoshi ED, Wang GT, Krafft GA, Erickson J: Novel fluorogenic substrates for assaying retroviral proteases by resonance energy transfer, Science 247: 954-958 (1989)].

The concentration of the synthetic peptide was 5.6 µM, while the final buffer conditions were: 50 mM tris, pH 7.5, 150 mM NaCl and 0.005% Brij35.

The kinetic test was carried out in the following manner: the fluorescence substrate was dissolved in 90 µl of reaction buffer. The kinetics of the conversion of the substrate by PCP were detected, in duplicate determinations, by measuring the fluorescence (ex. 355 nm/em. 538 nm) at from 0 to 120 min. The reaction was started by adding a suitable activity of PCP dissolved in 10 µl of reaction buffer. The emission, as a measure of the proteolytic activity, was measured at time intervals of initially 5 min and then subsequently at longer time intervals. The spontaneous decomposition of the fluorescence substrate in the added presence of reaction buffer lacking PCP activity was measured as the negative control. The fluorescence measurements took place at 37°C over a period of approx. 4 h. After the reaction had come to an end, the substrate was completely decomposed by adding proteinase K (Boehringer Mannheim, 1.44 µg per reaction mixture, dissolved in 10 µl of PBS) and incubating at 37°C for 20 min. The fact that the hydrolysis was complete was shown by there being no further increase in the fluorescence with time.

The relative conversion of substrate in % of the total quantity was calculated from:

$$\% \text{ conversion} = (F_t - F_{-t}) \div (F_{total} - F_{ini}) \times 100\%$$

where F_t is the relative fluorescence after a time interval t while incubating with PCP. F_{-t} is the corresponding relative fluorescence after the time interval t without PCP being added, F_{total} is the fluorescence following total hydrolysis produced by adding proteinase K, and F_{ini} is the initial relative fluorescence before starting the reaction by adding proteinase K.

In the test, the PCP activity used was typically adjusted such that approx. 20% of the substrate was converted by inhibited enzyme within the measurement period.

Figure 1 shows typical reaction kinetics (% conversion) which were obtained when adding the phosphinate inhibitor Z---PKF(PC)APL---O-Me.

The percentage inhibition obtained in the added presence of the inhibitors was calculated as follows:

% inhibition = 100% × % conversion (with inhibitor) ÷ % conversion (without inhibitor)

Figure 2 depicts the concentration-effect relationship of a phosphinate inhibitor.

Determining the specificity of PCP inhibitors by carrying out in-vitro investigations using the astacin-like protease meprin

Meprin is an enzyme which belongs to the astacin protease family and which is found in humans (Stöcker W, Gomis-Rüth FX, Bode W, Zwilling R: Implications of the three-dimensional structure of astacin for the structure and function of the astacin family of zinc-endopeptidases, Eur. J. Biochem. 214: 215-231 (1993)). The meprin activity test was carried out in a completely analogous manner to the in-vitro assay for measuring PCP activity. Instead of the recombinant PCP, use was made of human meprin, which also transforms the fluorescence-labeled decapeptide.

Figure 3 shows the specificity of the highly active phosphinate-peptide analog Z---PKF(PC)APL---O-Me for PCP. Even at concentrations of up to 100 nM, meprin is only slightly inhibited (in a dose-dependent manner) in its transformation of the fluorescence-labeled peptide.

Demonstration of biological activity

The biological activity of the substances can be demonstrated in cell culture assays and in vivo. For example, after administering the inhibitors, it is possible, in human

cell lines, to measure the decline in the concentration of free procollagen α_1(III) propeptide in the supernatants, because this peptide is released by PCP activity. The PIIICP concentrations in the supernatant can be measured using a recently established assay [cf. Burchardt ER, Schröder W, Heke M, Kohlmeyer J, Neumann R, Kroll W: Expression cloning of C-terminal procollagen (III) propeptide and its use in a novel serum assay to monitor liver fibrogenesis, Hepatology 26: 487A (1997)].

In order to demonstrate the antifibrotic effect of the substances in the liver, it is possible, for example, to use the animal model of acute [cf. Johnson SJ, Hines JE, Burt AD: Phenotypic modulation of perisinusoidal cells following acute liver injury: a quantitative analysis. Int. J. Exp. Path. 1992; 73: 765-772 (1992)] or chronic [cf. McLean E, McLean A, Sutton P: Instant Cirrhosis. An improved method for producing cirrhosis of the liver in rats by simultaneous administration of carbon tetrachloride and phenobarbitone, Br. J. Exp. Pathol. 1969; 50: 502-506 (1969)] carbon tetrachloride-induced liver damage, the model of liver fibrosis due to bile duct ligature [cf. Kountouras J, Billing B, Scheuer P: Prolonged bile obstruction: a new experimental model for cirrhosis in the rat, Br. J. Exp. Pathol. 1984; 65: 305-311 (1984)] or the liver fibrosis which is induced by heterologous serum [cf. Bhunchet E, Wake K.: Suppression of experimental hepatic fibrosis by administration of vitamin A, Lab. Invest. 1985; 52: 182-194 (1984)]. It is also possible to use other animal models in which liver fibrosis occurs for demonstrating the antifibrotic effect.

Depending on the organ in which the fibrosis is manifested, or on the nature of the fibrotic damage, it is also possible to use animal models for other manifestations of fibrosis, for example in the heart, in the kidneys, in the lungs, in the skin or in other organs.

The reduction in collagen deposition can be demonstrated, for example, by determining the content of hydroxyproline [cf. Gerling B, Becker M, Waldschmidt J, Rehmann M, Schuppan D.: Elevated serum aminoterminal procollagen type-III-peptide parallels collagen accumulation in rats with secondary biliary fibrosis,

Hepatology 1996; 25: 79-84 (1996)] in the fibrotic organs or by means of quantitative morphometry [cf. Kauschke SG, Knorr A, Olzen M, Burchardt ER: Expression of collagen (III) as determined by quantitative PCR and its correlation with extracellular collagen deposition in the rat CCl_4 model of liver fibrosis. Hepatology 26: 538A (1997)].

Figure 1 shows the transformation of the DABCYL-EDANS-decapeptide substrate by recombinant PCP as a function of time. The enzyme activity is almost completely inhibited in the presence of 100 nM inhibitor.

Figure 2 shows the concentration-effect relationship of the phosphinate inhibitor Z---PKF(PC)APL---O-Me.

Figure 3 shows the specificity of the highly active phosphinate-peptide analog Z---PKF(PC)APL---O-Me for PCP. Even at concentrations of up to 100 nM, meprin is only slightly inhibited, in a dose-dependent manner, in its transformation of the fluorescence-labeled peptide.

The present invention also includes pharmaceutical preparations which, in addition to inert, nontoxic, pharmaceutically suitable adjuvants and excipients, also comprise one or more compounds of the general formula (I), or which consist of one or more active compounds of the formulae (I), and also processes for producing these preparations.

In these preparations, the active compounds of the formulae (I) should be present at a concentration of from 0.1 to 99.5% by weight, preferably of from 0.5 to 95% by weight, of the total mixture.

In addition to the active compounds of the formulae (I), the pharmaceutical preparations can also comprise other pharmaceutical active compounds.

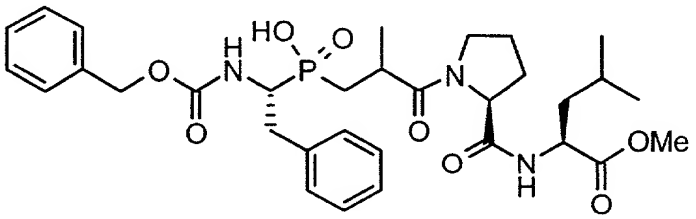
The abovementioned pharmaceutical preparations can be produced in a customary manner using known methods, for example using the adjuvant(s) or excipient(s).

In general, it has proved to be advantageous, in order to achieve the desired result, to administer the active compound(s) of the formulae (I) in total quantities of from about 0.01 to about 100 mg/kg, preferably in total quantities of from about 1 mg/kg to 50 mg/kg of body weight per 24 hours, where appropriate in the form of several individual doses.

However, it can, where appropriate, be advantageous to deviate from the said quantities, depending on the nature and body weight of the individual being treated, on the individual response to the drug, on the nature and severity of the disease, on the nature of the preparation and its administration, and on the time or time interval at which the administration is effected.

The preparation of the compounds of the formula (I) according to the invention is demonstrated below with the aid, by way of example, of an exemplary synthesis of the compound of the formula (I, R = methyl), to which the present invention is not, however, limited. Unless otherwise stated, all the quantity data given below refer to percentages by weight.

Ex. 1a: (1*R*)-1-{[(Benzyloxy)carbonyl]amino}-2-phenylethyl{3-[(2*S*)-2-({[(1*S*)-1-(methoxycarbonyl)-3-methylbutyl]amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid

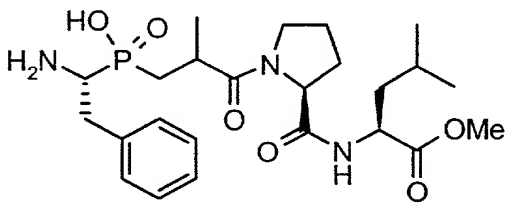


4.0 eq. of ethyldiisopropylamine, 1.50 eq. of benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBop) (J. Martinez et al., J. Med. Chem. 1988, 28, 1874; J. Costre, D. Le-Nguyen, B. Castro, Tetrahedron. Lett., 1990, 31, 2055) and, after from 2 to 5 min, 1.0 eq. of Pro-Leu-OMe-trifluoroacetic acid salt (prepared using standard methods of peptide chemistry, cf., e.g., Houben-Weyl, 4th edtn.: Methoden der Organischen Chemie (Methods in organic chemistry), Volume XVI-/-2, Synthese von Peptiden Teil 1 und Teil 2 (Synthesis of peptides, Part 1 and Part 2), Georg Thieme Verlag, Stuttgart 1974) are added, one after the other, at 0°C and under argon, to a solution of from 1.4 to 1.5 eq. of [(1-benzyloxycarbonyl)amino]-2-phenylethyl)-(2-carboxy-1-propyl)hydroxyphosphonic acid (prepared in analogy with WO 89/10961, p. 72, Ex. [14]) in absolute dichloromethane (from approx. 0.1 to 0.15 mol/l). After 15-30 min, the ice cooling is removed and the mixture is stirred overnight at room temperature. It is then diluted with dichloromethane and washed consecutively with saturated sodium hydrogen carbonate solution, 1N hydrochloric acid solution and saturated sodium chloride solution, dried over magnesium sulfate and concentrated in vacuo.

Yield: 133.7 mg of a yellowish oil as the crude product (no yield given since the product contains solvent residues)

LC-MS: rt (%), m/z (%) = 4.066 (34.6%), 630 (100, M+H); 4.197 (52.7), 630 (100, M+H).

Ex. 1b: (1*R*)-1-Amino-2-phenylethyl{3-[(2*S*)-2-({[(1*S*)-1-(methoxycarbonyl)-3-methylbutyl]amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid



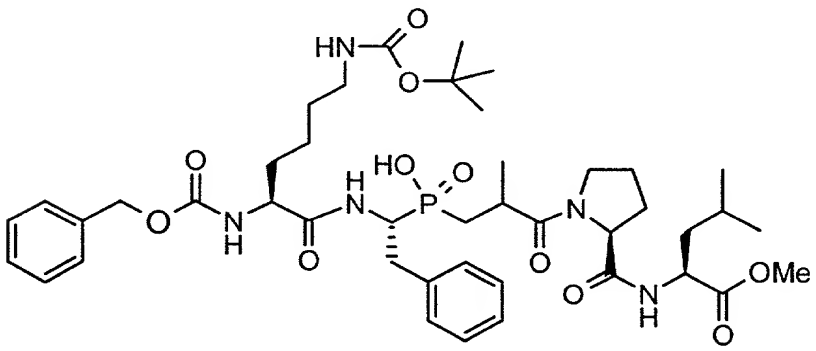
The protected amine derivative from Ex. 1a is dissolved in ethanol (approx. 0.05 mol/l), after which a catalyst (10% palladium on charcoal) is added to the solution under argon. The suspension is stirred vigorously at room temperature and under an H_2 atmosphere (normal pressure) for approx. 2 hours before being filtered through celite (subsequently washed thoroughly with ethanol). The filtrate is concentrated in vacuo and dried under high vacuum.

Yield: 80.4 mg (97.3% of theory)

LC-MS: rt, m/z (%) = 2.706, 496 (100, M+H)

HPLC: rt (%) = 5.80 (32.5), 5.91 (51.5).

Ex. 1c: (1*R*)-1-({(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-6-[(*tert*-butoxycarbonyl)amino]hexanoyl}amino)-2-phenylethyl{3-[(2*S*)-2-({[(1*S*)-1-(methoxycarbonyl)-3-methylbutyl]amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid

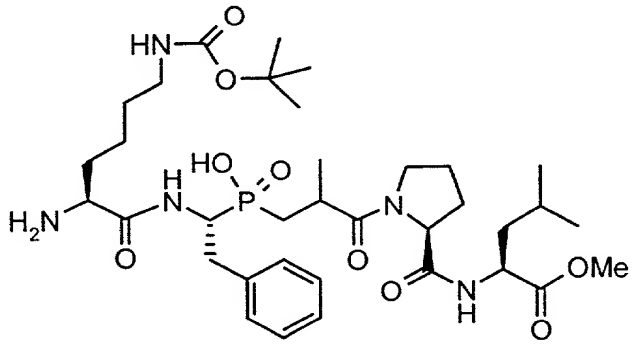


4.0 eq. of ethyldiisopropylamine, 1.50 eq. of PyBop and, after from 2 to 5 min, 1.45 eq. of Z-(NHBoc)-Lys-OH are added, one after the other, at 0°C and under argon, to a solution of 1.0 eq. of the compound from Ex. 1b. After 15-30 min, the ice cooling is removed and the mixture is stirred overnight at room temperature. It is diluted with dichloromethane and consecutively washed with saturated sodium hydrocarbonate solution, 1N hydrochloric acid solution and saturated sodium chloride solution, dried over magnesium sulfate and concentrated in vacuo.

Crude yield: 207 mg of a viscous oil, LC-MS: rt (%), m/z (%) = 4.456 (11.2%), 858 (100, M+H); 4.549 (32.7), 858 (100, M+H). The crude product is purified by means of preparative RP-HPLC.

Yield: 40 mg (32.8% of theory).

Ex. 1d: (1*R*)-1-({(2*S*)-2-Amino-6-[(*tert*-butoxycarbonyl)amino]hexanoyl}-amino)-2-phenylethyl{3-[(2*S*)-2-({[(1*S*)-1-(methoxycarbonyl)-3-methylbutyl]amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid

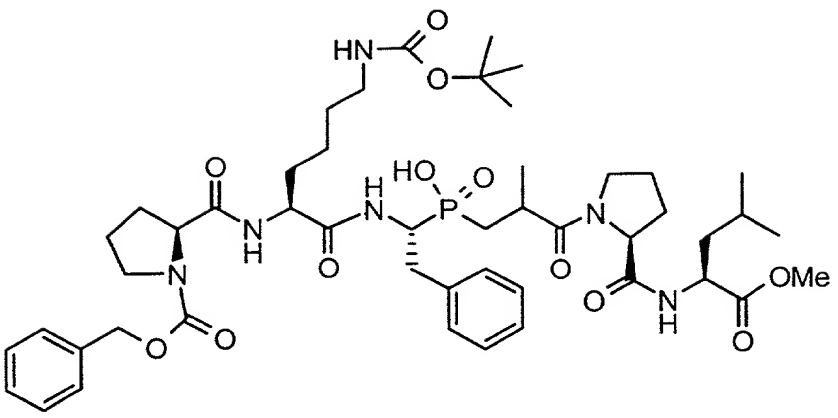


The protected amine derivative from Ex. 1c is dissolved in ethanol (approx. 0.05 mol/l), after which a catalyst (10% palladium on charcoal) is added to the solution under argon. The suspension is stirred vigorously at room temperature and under an H_2 atmosphere (normal pressure) for approx. 2 hours before being filtered through celite (subsequently washed thoroughly with ethanol). The filtrate is concentrated in vacuo and dried under high vacuum.

Yield: 31 mg (85.4% of theory)

LC-MS: rt (%), m/z (%) = 2.97 (85.8%), 724 (100, M+H).

Ex. 1e: (1*R*)-1-({(2*S*)-2-Amino-6-[(*tert*-butoxycarbonyl)amino]hexanoyl}-amino)-2-phenylethyl{3-[(2*S*)-2-({[(1*S*)-1-(methoxycarbonyl)-3-methylbutyl]amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid



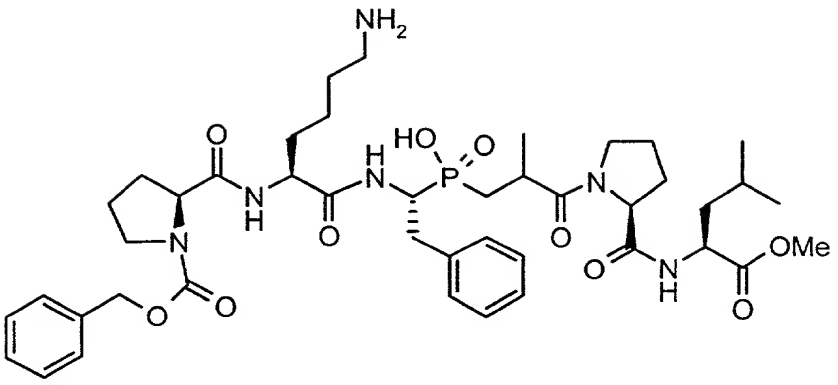
4.0 eq. of ethyldiisopropylamine, 1.50 eq. of PyBop and, after from 2 to 5 min, 1.45 eq. of Z-Pro-OH are added, one after the other, at 0°C and under argon, to a solution of 1.0 eq. of the compound from Ex. 1d. After 15-30 min, the ice cooling is removed and the mixture is stirred overnight at room temperature. It is diluted with dichloromethane and washed consecutively with saturated sodium hydrocarbonate solution, 1N hydrochloric acid solution and saturated sodium chloride solution, dried over magnesium sulfate and concentrated in vacuo. 50 mg of crude product are obtained.

LC-MS: rt (%), m/z (%) = 4.394 (25.9%), 955 (100, M+H); 4.509 (44.6), 955 (100, M+H). The crude product is purified by means of preparative RP-HPLC.

Yield: 24.5 mg (59.9% of theory).

Ex. 1f: Preparation of the compound of the formula (I), R = methyl

(1*R*)-1-({(2*S*)-6-amino-2-[({(2*S*)-1-[(benzyloxy)carbonyl]tetrahydro-1*H*-pyrrol-2-yl}carbonyl)amino]hexanoyl}amino)-2-phenylethyl{3-[(2*S*)-2-({[(1*S*)-1-(methoxycarbonyl)-3-methylbutyl]amino}carbonyl)tetrahydro-1*H*-pyrrol-1-yl]-2-methyl-3-oxopropyl}phosphinic acid



0.3 ml of trifluoroacetic acid is added dropwise to 24.50 mg (0.03 mmol) of the compound from Ex. 1e in an ice-cooled mixture consisting of 0.3 ml of methylene chloride and 0.03 ml of water. The cooling is removed and the mixture is stirred at room temperature for two hours before being concentrated in vacuo and the residue being thoroughly dried under high vacuum.

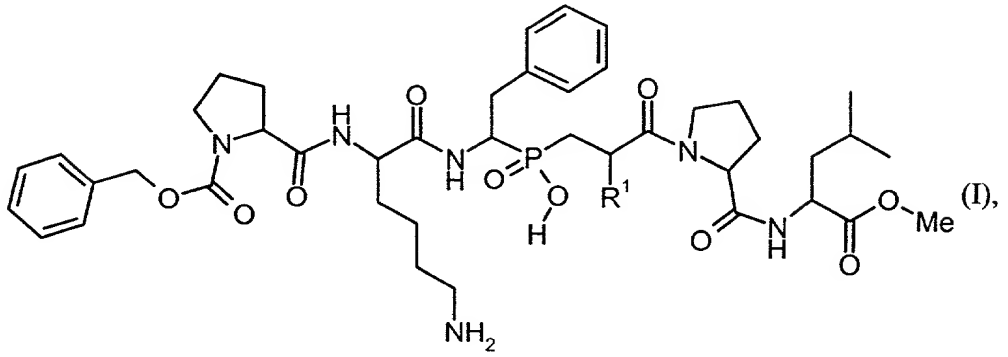
Yield: 21.0 mg of a colorless solid (95.8% of theory)

LC-MS: rt, m/z (%) = 3.057 (both stereoisomers), 855 (100, M+H)

RP-HPLC rt (%) = 2.18 (48.7), 2.42 (46.0).

Patent Claims

1. Use of phosphinate-peptide analogs of the general formula (I)



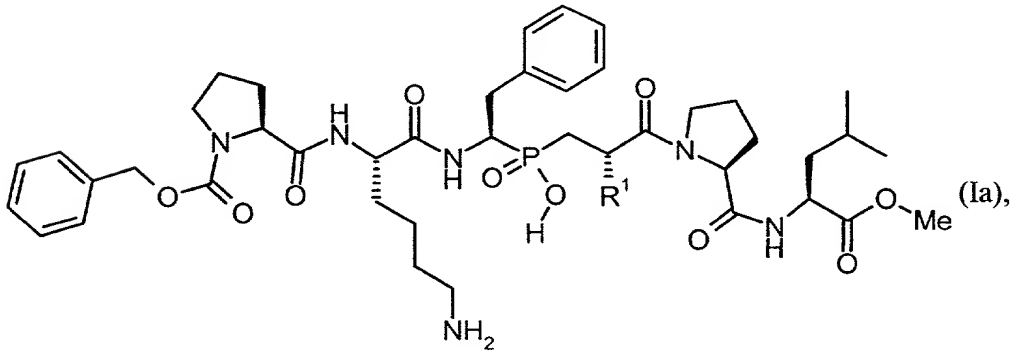
(I),

in which

R^1 represents hydrogen or methyl,

and/or their stereoisomers and salts for producing drugs for treating fibrotic diseases.

2. Use according to Claim 1, characterized in that compounds of the general formula (I) having the configuration shown in formula (Ia)



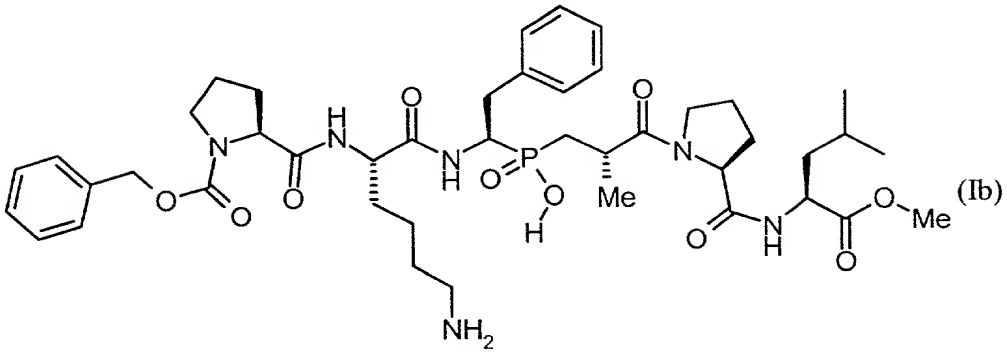
(Ia),

in which

R^1 represents hydrogen or methyl,

and/or their stereoisomers and salts are used.

3. Use according to Claim 1, characterized in that the compound of the formula (Ib)



(Ib)

and/or its enantiomers and salts is/are used.

4. Use according to Claim 1 for treating liver fibrosis.

Figure 1

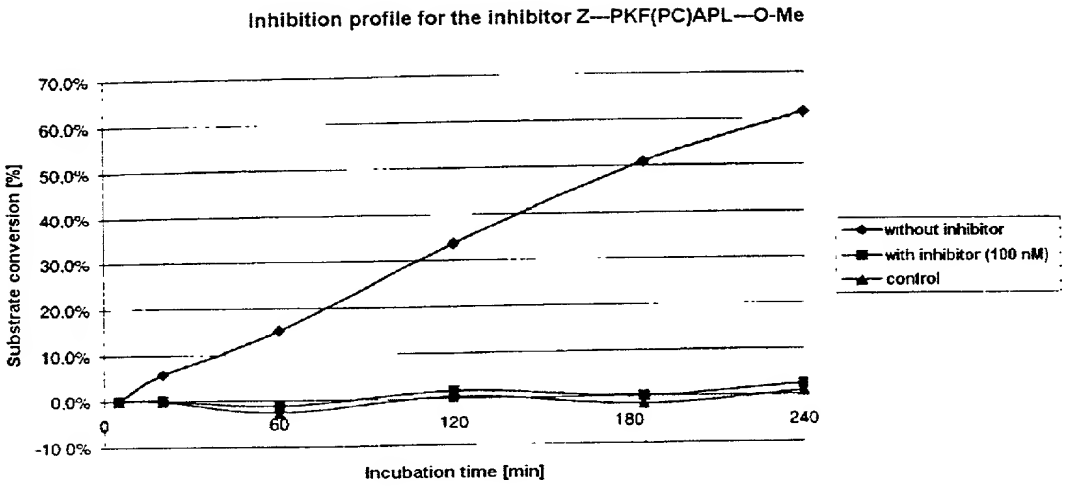

Inhibition profile for the inhibitor Z---PKF(PC)APL---O-Me
Substrate conversion [%]
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
-10.0%
0
60
120
180
240
Incubation time [min]
without inhibitor
with inhibitor (100 nM)
control

Figure 2

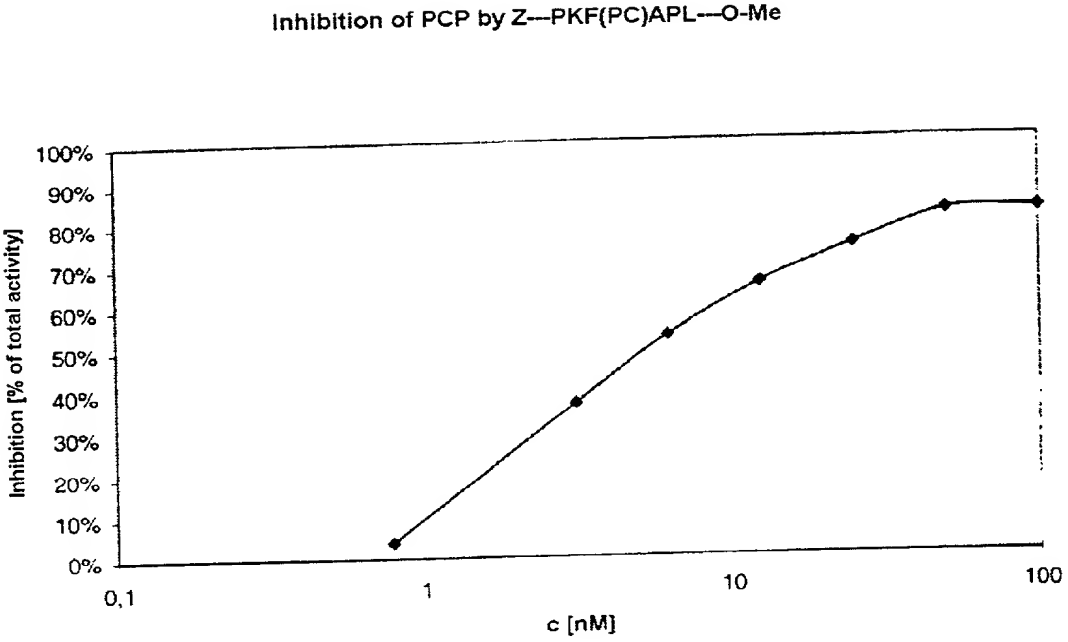
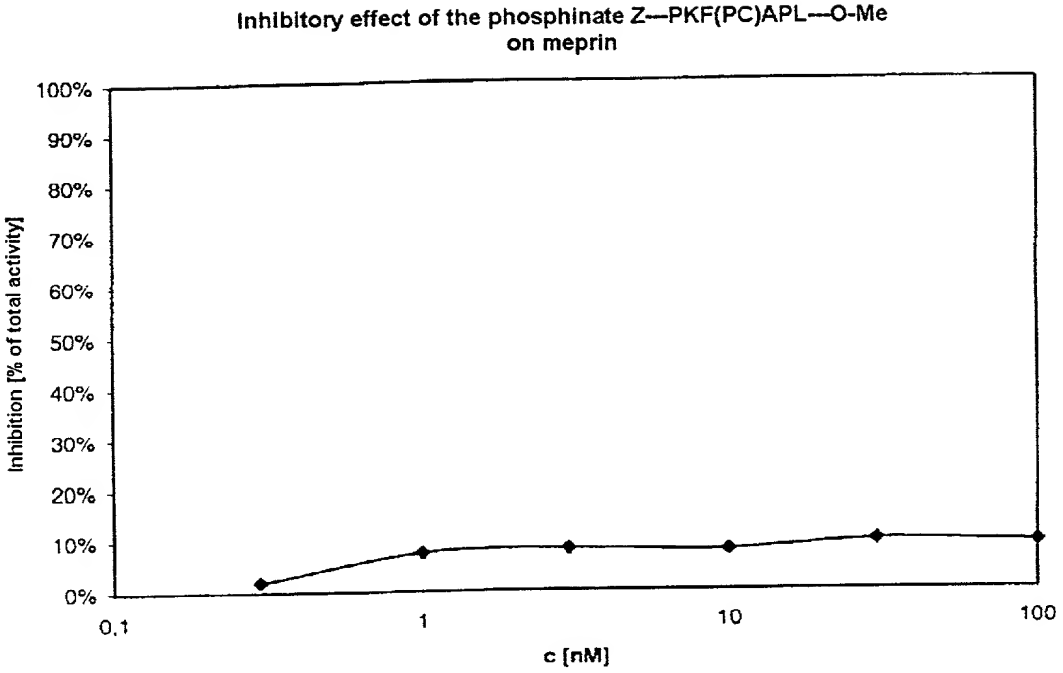

Inhibition of PCP by Z---PKF(PC)APL---O-Me
Inhibition [% of total activity]
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0,1
1
10
100
c [nM]

Figure 3


Inhibitory effect of the phosphinate Z---PKF(PC)APL---O-Me on meprin
Inhibition [% of total activity]
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0.1
1
10
100
c [nM]

COMBINED DECLARATION AND POWER OF ATTORNEY	ATTORNEY DOCKET NO Le A 33 256

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought
on the invention entitled

PHOSPHINATE PEPTIDE ANALOGS FOR THE TREATMENT OF FIBROTIC DISORDERS

the specification of which is attached hereto,

or was filed on **October 28, 1999**

as a PCT Application Serial No. **PCT/EP99/08181**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s), the priority(ies) of which is/are to be claimed:

198 50 072.6	**Germany**	**October 30, 1998**
(Number)	(Country)	(Month/Day/Year Filed)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose the material information as defined in Title 37, Code of Federal Regulations, §1.56 which occured between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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FULL NAME OF FIFTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SIXTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SEVENTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		